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Proximate and Preliminary Phytochemical Analysis of Fruits of C. Equisetifolia and Seeds of P. Longifolia

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Abstract: Fruit of C. Equisetifolia and P. Longifolia (Ashoka) has been used in the traditional method of medicine for treatment of various sicknesses. The use of extracts of plant and their isolated compound/s has offered basis in the research work of modern pharmaceutical medicines. C. Equisetifolia & P. Longifolia is a evergreen tree, naturally found in India and has been identified to possess antifungal, anti-ulcer, antioxidant, anti-inflammatory and antimicrobial activities. The primary studies of fruit of C. Equisetifolia & seeds of P. Longifolia have been accomplished to scrutinize its potentialities. The primary evaluation of phytochemical study of various extracts indicated that the seeds are rich source of tannins, alkaloids, flavonoids phenols, carbohydrates and fats. This study offers essential data on the accessibility of various chemical ingredients present in fruit of C. Equisetifolia and P. longifolia seeds. At the same time Loss on dehydrating the material and experiment on moisture content was supported to know the existence of volatile organic substance
Key words: Fruit of C. Equisetifolia, Phytochemical parameters, Polyalthia longifolia seeds.

I. INTRODUCTION

C. Equisetifolia (Casuarinaceae) is a deciduous tree that occurs in open, coastal habitats including sand beaches, rocky coasts and sand dunes. It is native to Australia and Southeast Asia and introduced into Florida. It is also called as Australian pine tree or whistling pine tree. P. longifolia, (Annonaceae) is mainly found in India and Sri Lanka. It is known by several common names like the Buddha, Ashoka, Tree and Indian Fir tree, Ashoka or Devadaru in Sanskrit, Debdaru in Bengali. The literature survey shows that various parts of plant hold medicinal value. In case of fruit of C. Equisetifolia and seeds of P. Longifolia extracts & secluded complex/s has supply foundation in the research of up to date pharmaceutical drugs. A quantity of organically energetic complexes has been inaccessible as of this plant. The extract of plant & inaccessible complexes has been deliberated for a variety of organic performances like as, antifungal[14], antibacterial[1-5], anti-inflammatory, anticancer[4] hypotensive[11]& cytotoxic[13], analgesic[13]and fungicides[13]. Taking into consideration the importance of medicinal value of the plant, screening of these valued plants – C. Equisetifolia fruit and P. Longifolia seed was attained. In the existing study an effort is made to explore the primary proximate and phytochemical analysis to supports modern chemical inventions

II. MATERIAL AND METHODS

Fruits of C. Equisetifolia and seeds of P. Longifolia is collected in the month of August in Pune, Maharashtra, India. Seeds of P. Longifolia were authenticated at Botanical Survey of India, Pune, India. Its Voucher Specimen No. is BSI/WRC/Tech/2009/POLMK1.The dried and crushed material was used for analysis.

III. PROXIMATE ANALYSIS

Proximate analysis of C. Equisetifolia Fruits and seeds of P. Longifolia

1.0 g of stable mass of fruit of C. Equisetifolia and seeds of P. Longifolia is taken separately in silica crucible situated in an oven retained at 110°C to conclude the amount of moisture. Also ash was calculated by the ignition of 1.0 g dehydrated tests sited in a muffle furnace uphold at 750°C for 6 Hrs, At the same time 3.0 g of the dehydrated test in a Soxhlet device with the help of pet- ether (50-80°C) as the extracting unfinished fat was achieved by methodically extract. Basic protein (total % nitrogen x 6.25) was found out by the method Kjeldhal, with the help of 2.0 g of dehydrated, defatted tests. By getting the difference Carbohydrate substance was verified.

Parameter	**Percentage (%)**	
	Seeds of P. Longifolia	**Fruits of C. Equisetifolia**
Moisture	4.0 ± 0.2	7.15 ± 0.2
Volatile Mater	-	-
Dry matter	85.0 ± 0.1	1.35 ± 0.1
Crude fat	6.5 ± 0.2	3.82 ± 0.2
Protein	12.0 ± 0.3	3.07 ± 0.3
Crude fiber	5.3 ± 0.30	16.62 ± 0.30
Total carbohydrate	64.3 ± 0.20	64.78 ± 0.20

Table 1: Proximate analysis of P. Longifolia & C. Equisetifolia

IV. PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of C. Equisetifolia Fruits and seeds of P. Longifolia

Air shade dried out & powdered adsorber (1g), was kept in interaction with various polar and non-polar solvents at R.T. The powdered plant samples were extracted consecutively with Hexane, ethanol, ethyl acetate, methanol & water using apparatus of Soxhlet at temperature 55-85 °C and for 8-10 hrs in order to extract the non-polar & polar compounds[10]. For every solvent extraction, the powdered pack material was air dried and then used. The respective solvents extracts were reduced at R.T. & stored at 4°C for further use. The dried plant extracts were then re-dissolved in dimethyl sulfoxide and to get the solution of 10 mg/10 mL for each extract. Following tests were carried out for phytochemical analysis[14]. Results are mentioned in the table given below.

- Steroids (Salkowski Test): 2ml extract + 2ml $CHCl_3$ + 2ml H_2SO_4 (conc.) → Reddish brown ring at the junction
- Alkaloids (Hager's Test): 2ml extract + few drops of Hager's reagent → Yellow precipitate
- Flavonoids: 1ml extract + 1ml $Pb(OAc)_4$ (10%) → Yellow coloration
- Tannins (Braymer's Test) 2ml extract + 2ml H_2O + 2-3 drops $FeCl_3$ (5%) → Green precipitate
- Proteins (Xanthoproteic Test): 1ml extract + 1ml H_2SO_4(conc.) →White precipitate
- Phenol: 1ml extract + 1ml $FeCl_3$ → a red colour
- Saponins (Foam Test) : 5ml extract + 5ml H2O + heat → Froth appears
- Carbohydrates (Molisch's Test): 2ml extract + 10ml H_2O + 2 drops Ethanolic α□naphthol (20%) +2ml H_2SO_4 (conc.) →Reddish violet ring at the junction

Chemical Constituents	**Seed Extract P. Longifolia**				
	Hexane	**E. Acetate**	**Acetone**	**Ethanol**	**Methanol**
Steroid	+ ve	+ ve	+ ve	+ ve	+ ve

Alkaloid	- ve	- ve	+ ve	+ ve	+ ve
Phenol	+ ve	+ ve	- ve	- ve	+ ve
Flavonoid	+ ve	+ ve	+ ve	+ ve	+ ve
Tannin/s	+ ve	+ ve	+ ve	+ ve	+ ve
Protein	- ve	- ve	+ ve	+ ve	+ ve
Starch	- ve	- ve	+ ve	+ ve	+ ve

Table 2 : Seed extract of P. Longifolia (+ Ve = present, - Ve = absent)

Chemical Constituents	**Fruit of C. Equisetifolia**
	Ethanol
Steroid	+ ve
Alkaloid	+ ve
Glycosides	+ ve
Flavonoid	+ ve
Tannin/s	+ ve

Table 3 : Fruit extract of C. Equisetifolia (+ Ve = present, - Ve = absent)

Extracts	**Percentage Value**
Hexane	6.40%
Ethyl acetate	6.45%
Acetone	8.0%
Ethyl alcohol	9.3%
Methyl alcohol	10.0%
Aqueous	11.5%

Table 4: Extractive values

Parameters	**Value**
Moisture Content	9.12%
pH	6.06%
Loss on drying	13.18%
Total ash	3.16%
Acid insoluble matter	12.8%
Water soluble matter	11.6%

Table 5: Analysis of phytochemical parameters

V. ANALYSIS OF AMINO ACIDS

The amino acids are basic units of protein. They have important role in the metabolic pathways for synthesis of secondary metabolites therefore, their presence was detected[3-7]. Amino acids from the seeds of P. Longifolia and C. Equisetifolia were carried out by using C_2H_5OH, H_2O and saline extract. Various different mobile phases were employed for paper chromatography technique. Three mobile phases were selected as different amino acids were detected from different mobile phases. Those were

Mobile Phase 1: n-butanol: ethanol: water (2:2:1)

Mobile Phase 2: n-butanol: ethanol: water: Pyridine (2:0.5:1:1.5)

Mobile Phase 3: Iso Propyl Alcohol: Water: Acetic acid (5:4:1)

Sr. No.	Amino Acids	Std. Rf	Obs. Rf	Obs. Rf
			C. Equisetifolia	P. Longifolia
1	DL-Alanine	0.221	0.219	0223
2	DL-Aspartic acid	0.073	0.072	0.079
3	L-Cysteine Hydrochloride	0.336	0.334	0.338
4	DL-Serine	0.139	0.136	0.130
5	L-Glutamic acid	0.300	0.297	0.310
6	L-Arginine monohydrochloride	0.212	0.210	0.214
7	L-Lysine monohydrochloride	0.081	0.75	0.080
8	DL-Threonine	0.350	0.345	0.340

Table 6 : Amino acids in Mobile Phase 1 for water extract

Sr. No.	Amino Acids	Std. Rf	Obs. Rf	Obs. Rf
			C. Equisetifolia	P. Longifolia
1	L-Lysine monohydrochloride	0.081	0.075	0.078
2	L-histidine monohydrochloride	0.057	0.050	0.060
3	L-Arginine monohydrochloride	0.212	0.210	0.208
4	DL-Alanine	0.221	0.216	0.215
5	DL-Threonine	0.350	0.348	0.347
6	DL-Serine	0.139	0.130	0.135

Table 7 : Amino acids in Mobile Phase 2 for ethanol extract

Sr. No.	Amino Acids	Std. Rf	Obs. Rf	Obs. Rf
			C. Equisetifolia	P. Longifolia
1	DL-2-Amino-N-Butyric acid	0.891	0.885	0.880
2	DL-Alanine	0.221	0.213	0.212
3	DL-Valine	0.612	0.601	0.617
4	DL-Methionine	0.065	0.058	0.053
5	DL-Serine	0.220	0.200	0.211

6	L-Lysine Monohydrochloride	0.092	0.090	0.090
7	DL- Threonine	0.617	0.615	0.600

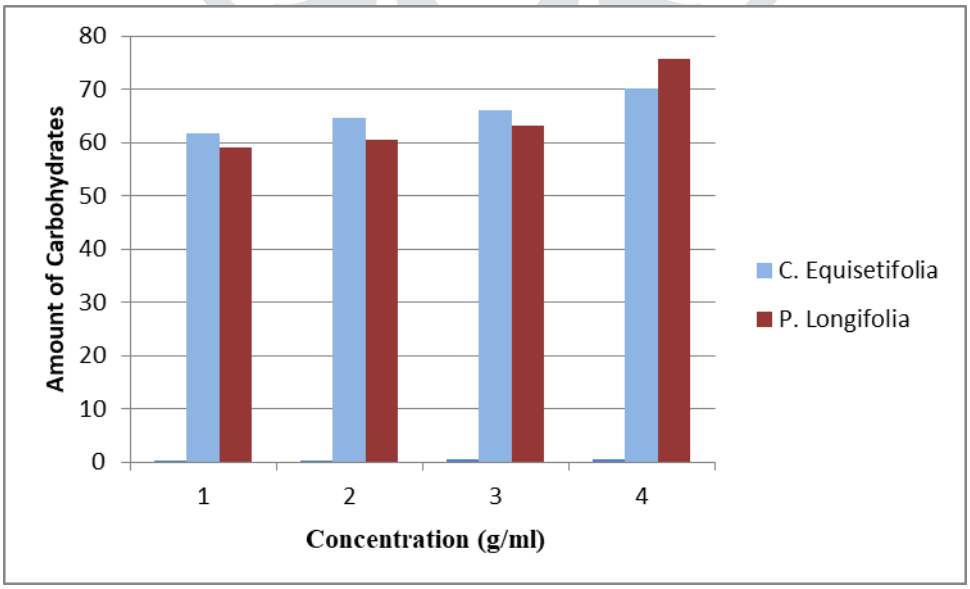
Table 8 : Amino acids in Mobile Phase 3 for Saline extract

VI. ANALYSIS OF CARBOHYDRATES

Carbohydrates play very important role in plant metabolism. Quantitative determination of carbohydrates from fruit of C. Equisetifolia and seed of P. Longifolia extract has been carried out by phenol-sulphuric acid method. The absorbance is measured by UV-Visible Spectrophotometer at 470 nm. Amount of carbohydrate(mg/ml) is calculated as mentioned table.

No.	**Conc. (g/ml)**	**Absorbance**		**Amount of Carbohydrates (mg/ml)**	
		C. Equisetifolia	**P. Longifolia**	**C. Equisetifolia**	**P. Longifolia**
1	0.1	0.45	0.43	61.813	59.066
2	0.2	0.47	0.44	64.572	60.451
3	0.4	0.48	0.46	65.969	63.221
4	0.6	0.51	0.55	70.146	75.647

Table 9: Amount of Carbohydrate



The amount of carbohydrate is increased by increasing the concentration of extract.

VII. CONCLUSION:

The present study confirms the use of fruits of C. Equisetifolia and seeds of P. Longifolia seeds in traditional medicines and phytochemical data will be helpful in the standardization and quality control of precious indigenous drug and also for pharmaceutical industries.

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